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Effects of Pentachlorophenol on Hepatic Drug-Metabolizing Enzymes and Porphyria Related to Contamination with Chlorinated Dibenza-p-Dioxins and Dibenzofurans

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16. ABSTRACT

The hepatic effects of technical and pure grade pentachlorophenol were investigated in female rats fed 20, 100 and 500 ppm of each for 8 months. Technical pentachlorophenol was contaminated with 8 ppm hexa-, 520 ppm hepta-, and 1380 ppm octachlorodibenzodioxins; pure pentachlorophenol contained less than 0.1 ppm of each of these contaminants. Technical pentachlorophenol produced hepatic porphyria and increased hepatic aryl hydrocarbon hydroxylase activity, glucuronyl transferase activity, liver weight, cytochrome P-450 and microsomal heme, but not N-demethylase activity. The peak of the CO-difference spectrum of cytochrome P-450 was shifted to 448 nm, and there was a dramatic increase in the 455-430 ratios of the ethyl isocyanide difference spectrum. The enzyme changes were observed at 20 ppm of technical pentachlorophenol. Porphyria occurred at 100 and 500 ppm. Pure pentachlorophenol had no significant effect on aryl hydrocarbon hydroxylase activity, liver weight, cytochrome P-450, microsomal heme, the ethyl isocyanide difference spectrum or N-demethylase activity at any dose level, but did increase glucuronyl transferase at 500 ppm. In contrast, both pure and technical pentachlorophenol decreased body weight gain comparably at 500 ppm. It is concluded that technical pentachlorophenol produces a number of liver changes which cannot be attributed to pentachlorophenol itself, but are consistent with the effects of biologically active chlorinated dibenzo-p-dioxins and dibenzofurans.

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EFFECTS OF PENTACHLOROPHENOL ON HEPATIC DRUG-METABOLIZING ENZYMES AND PORPHYRIA RELATED TO CONTAMINATION WITH CHLORINATED DIBENZO-*p*-DIOXINS AND DIBENZOFURANS*

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Abstract—The hepatic effects of technical and pure grade pentachlorophenol were investigated in female rats fed 20, 100 and 500 ppm of each for 8 months. Technical pentachlorophenol was contaminated with 8 ppm hexa-, 520 ppm hepta-, and 1380 ppm octachlorodibenzodioxins and with 4 ppm tetra-, 42 ppm penta-, 90 ppm hexa-, 1500 ppm hepta- and 200 ppm octachlorodibenzofurans; pure pentachlorophenol contained less than 0.1 ppm of each of these contaminants. Technical pentachlorophenol produced hepatic porphyria and increased hepatic aryl hydrocarbon hydroxylase activity, glucuronyl transferase activity, liver weight, cytochrome P-450 and microsomal heme, but not *N*-demethylase activity. The peak of the CO-difference spectrum of cytochrome P-450 was shifted to 448 nm, and there was a dramatic increase in the 455–430 ratios of the ethyl isocyanide difference spectrum. The enzyme changes were observed at 20 ppm of technical pentachlorophenol. Porphyria occurred at 100 and 500 ppm. Pure pentachlorophenol had no significant effect on aryl hydrocarbon hydroxylase activity, liver weight, cytochrome P-450, microsomal heme, the ethyl isocyanide difference spectrum or *N*-demethylase activity at any dose level, but did increase glucuronyl transferase at 500 ppm. In contrast, both pure and technical pentachlorophenol decreased body weight gain comparably at 500 ppm. It is concluded that technical pentachlorophenol produces a number of liver changes which cannot be attributed to pentachlorophenol itself, but are consistent with the effects of biologically active chlorinated dibenzo-*p*-dioxins and dibenzofurans.

Pentachlorophenol is widely used as a wood preservative by the lumber industry, and as a fungicide and a bactericide. Recently, commercial preparations of pentachlorophenol have been shown to be contaminated with hexa-, hepta- and octachlorodibenzo-*p*-dioxins and dibenzofurans [1]. Chlorinated dibenzo-*p*-dioxins, contaminants of a number of industrial products, are formed when alkali metal salts of chlorinated phenols are heated at high temperatures [2]. These compounds, especially 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), are among the most potent and toxic compounds known. The oral LD_{50} of TCDD is 1 μg/kg in guinea pigs and 100 μg/kg in rats [3]. TCDD produces fetal malformations, hepatic necrosis, edema, thymic atrophy, hyperkeratosis in the rabbit ear and a number of other biological responses [4]. Hepatic effects of TCDD include induction of δ-aminolevulinic acid (ALA) synthetase in the chick embryo [5], hepatic porphyria [6], and increases in cytochrome P-448 and activity of a number of drug-metabolizing enzymes [7, 8]. Hexachlorodibenzo-*p*-dioxins also produce fetal malformations and pathology at low doses [9]. Less is known about the toxicity of other chlorinated dibenzo-*p*-dioxins and dibenzofurans.

Because of the extreme potency of the chlorinated dibenzo-*p*-dioxins, much of the toxicity of many technical grade chlorinated aromatic chemicals could be due to these contaminants. The chloracne and porphyria cutanea tarda reported in a 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) factory [10] have been attributed to contamination by TCDD [11]. Chloracne was also observed in workers in a factory in West Germany which produced technical pentachlorophenol [12]. Technical pentachlorophenol produced in this factory produced hyperkeratosis in the rabbit ear while pure pentachlorophenol did not [13], suggesting the presence of chlorinated dibenzo-*p*-dioxins. In the late 1950s, the presence of chlorinated dibenzo-*p*-dioxins in feed additives was responsible for millions of deaths in broiler chicks, and 2,4,5-trichlorophenol and pentachlorophenol were suggested as the probable sources [14, 15]. A number of chlorinated benzenes produce porphyria in experimental animals [16]. The possible contamination of these products by chlorinated dibenzo-*p*-dioxins is unknown.

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However, hexachlorobenzene, a fungicide responsible for a widespread accidental outbreak of human cutaneous porphyria in Turkey in 1959 [17] is reported to be contaminated with octachloro-*p*-dibenzodioxin, and hepta- and octachlorodibenzofurans [18].

The effects of pentachlorophenol on the liver have received little attention, despite its wide usage, structural similarity to the porphyrogenic chlorinated benzenes and 2,4,5-T, and contamination with hexa- and octachlorodibenzo-*p*-dioxins. Knudsen *et al.* [19] reported that 200 ppm of a technical grade of pentachlorophenol increased aniline hydroxylase activity slightly but had no effect on *N*-demethylase activity. Kimbrough and Linder [20] reported histological changes in livers of rats fed a technical grade of pentachlorophenol that were not seen when rats were fed pure grade pentachlorophenol.

The present study was undertaken to determine the effects of pentachlorophenol on the liver and to distinguish its effects from those of its contaminants by comparing the effects of pure and technical grades of pentachlorophenol, with known composition of chlorinated dibenzo-*p*-dioxins and dibenzofurans, on the development of hepatic porphyria and alterations in hepatic drug-metabolizing enzymes.

METHODS

Animals. Forty-two female Sherman rats (29- to 33-days-old) were randomly distributed into seven groups of six each and fed ground Purina laboratory chow *ad lib.* containing 0, 20, 100 or 500 ppm of pure pentachlorophenol or 20, 100 or 500 ppm of technical grade pentachlorophenol. Rats were ear tagged and body weights and clinical symptoms recorded weekly. Food consumption was measured during weeks 1, 2, 5, 9, 15, 20, 25 and 30. Urine was collected for 24 hr in metabolic cages at 4, 8, 16, 24 and 31 weeks. Rats were sacrificed by decapitation at 8 months, and ALA synthetase, aryl hydrocarbon hydroxylase, aminopyrine *N*-demethylase, *p*-nitrophenol glucuronyl transferase, ethyl isocyanide difference spectra, cytochrome P-450, total microsomal heme and hepatic porphyrins determined as described below.

Chemicals. Technical pentachlorophenol (86 per cent pure, lot KA578) from Monsanto Chemical Co. was labeled 10 per cent other chlorophenols and 4 per cent inert ingredients. Pure pentachlorophenol (>99 per cent pure) was from Aldrich Chemical Co.

Chemical analysis. Both pentachlorophenol samples were analyzed for chlorodibenzo-*p*-dioxins by a method similar to that of Crummett and Stehl [21]. The pentachlorophenol was dissolved in benzene and transferred to sea sand (Fisher S-25, washed and ignited) and the solvent evaporated. The sand was then transferred quantitatively to an alumina column (Fisher A-540). The column was eluted with 150 ml hexane and then 100 ml of 20% CH_2Cl_2/hexane. Recovery studies showed 80–98 per cent of the dioxins to be in the second fraction. Quantitation was accomplished by selected ion monitoring gas chromatography–mass spectrometry (GC–MS) of the two most abundant ions in the respective molecular ion clusters. A Finnigan 9500 gas chromatograph is interfaced by a glass jet separator to a Finnigan 3300/F mass spectrometer equipped with an electron impact source. Mass spectrometer control and data acquisition were accomplished by means of a Finnigan 6100 data system. Peak areas were determined using the standard software. The instrument response was determined by measuring standard curves for octa-, 1,2,3,4,6,7,9-hepta-, 1,2,3,4,6,7,8-hepta-, and 1,2,3,6,7,8-hexachlorodibenzo-*p*-dioxins and for 2,3,7,8-tetra-, 2,3,4,7,8-penta-, and octachlorodibenzofurans. The instrument response to the penta-isomer was used to determine the amount of hexachlorodibenzofurans, and the response to the octa-isomer was used to determine the amount of heptachlorodibenzofurans. The GC column was 2 mm i.d. × 5 ft containing 3% OV210 on 100/120 mesh Gas Chrom Q (Applied Sciences Laboratories).

The purity of technical pentachlorophenol was confirmed after derivitization with *N,O-bis*-(trimethylsilyl)-acetamide (Pierce Chemical Co., Rockford, Ill.) using a Hewlett–Packard 5700 A gas chromatograph equipped with dual flame ionization detectors and a 1/8 in × 2 m, 10% OV101 column operated at 130° with an He flow of 25 ml/min. Instrument response for pentachlorophenoltrimethylsilylsilylether was deter-

Table 1. Chemical analysis of pure and technical pentachlorophenol

Source	Pentachlorophenol: Pure (Aldrich, lot 120,717)	Pentachlorophenol: Technical (Monsanto, lot KA578)
Phenols*		
Pentachlorophenol	>99%	84.6%
Tetrachlorophenol	<0.1 ppm	3%
Nonphenolics (ppm)*		
Dibenzo-*p*-dioxins		
Tetrachloro-	<0.1 ppm	<0.1 ppm
Pentachloro-	<0.1	<0.1
Hexachloro-	<0.1	8
Heptachloro-	≤0.1	520
Octachloro-	≤0.1	1380
Dibenzofurans		
Tetrachloro-	<0.1	≤4
Pentachloro-	<0.1	40
Hexachloro-	<0.1	90
Heptachloro-	<0.1	400
Octachloro-	<0.1	260

* Samples were analyzed by GC–MS. The lower detection limit was 0.1 ppm.

mined with similarly derivatized pure (> 99 per cent) pentachlorophenol.

Assays. ALA synthetase was assayed in whole liver homogenates [22], aminopyrine *N*-demethylase in 9000 *g* supernatants [23] with the substitution of HEPES for Tris buffer, and aryl hydrocarbon hydroxylase in 9000 *g* supernatants [24]. Microsomes were prepared by $CaCl_2$ precipitation [25], resuspended in 1.15% KCl containing 0.1 M K_2HPO_4 buffer, pH 7.4, and 25% glycerol, and stored at 0° for 24–48 hr for determination of cytochrome P-450. *p*-Nitrophenol glucuronyl transferase was assayed in microsomes equivalent to 50 mg wet wt of liver (resuspended in 1.15% KCl) incubated for 5 min in the presence of 0.3% digitonin, 0.9 mM *p*-nitrophenol, and 2.7 mM UDPGA as described for *o*-aminophenol [26]. *p*-Nitrophenol was determined spectrophotometrically [27]. Microsomal cytochrome P-450, protoheme [28, 29] and ethyl isocyanide ratios [30] were determined using an Aminco DW-2 spectrophotometer. The ethyl isocyanide difference spectrum was obtained in 0.1 M potassium phosphate buffer, pH 7.4, by addition of 10 μl of a 0.33 M solution of ethyl isocyanide to 3 ml of the microsomal suspension of dithionite-reduced microsomes. The final ligand concentration (1.1 mM) was saturating, because addition of more ethyl isocyanide produced no greater absorbance. Microsomal protein [31], urinary porphyrin [32], and urinary ALA and porphobilinogen (PBG) [33] were measured as previously described. Tissue porphyrins were determined by the method of Abbritti and De Matteis [34] using uroporphyrin as a standard. The porphyrins in selected liver samples were separated and identified by thin-layer chromatography [35].

Data were analyzed by analysis of variance followed by Duncan's multiple-range test at $P = 0.05$ [36].

RESULTS

Table 1 shows the results of GC–MS analysis of both samples of pentachlorophenol. Technical pentachlorophenol was contaminated with 1380 ppm octa-, 520 ppm hepta- and 8 ppm hexachlorodibenzo-*p*-dioxins and with 260 ppm octa-, 400 ppm hepta-, 90 ppm hexa-, 40 ppm penta- and approximately 4 ppm tetrachlorodibenzofurans. Pure pentachlorophenol contained less than 0.1 ppm of all of the above isomers. Tetrachlorodibenzo-*p*-dioxins were not detected in either sample with a detection limit of 0.1 ppm. Although tetrachlorodibenzofurans were detected in technical pentachlorophenol, the 2,3,7,8-isomer was not present. Approximately one-half the total amount of hexachlorodibenzo-*p*-dioxins present in technical pentachlorophenol was the 1,2,3,6,7,8-hexa-isomer. Two additional unidentified hexa-isomers were present. 1,2,3,4,7,8-Hexa- and 1,2,3,7,8,9-hexa-isomers were absent. 1,2,3,4,6,7,8-Hepta(160 ppm)- and 1,2,3,4,6,7,9-hepta(360 ppm)-isomers were identified in technical pentachlorophenol. Chlorodiphenyl ethers were detected, but these were not quantitated. Technical pentachlorophenol was found to contain 84.6 per cent pentachlorophenol. The only other chlorophenol detected by GC–MS was 2,3,4,6-tetrachlorophenol (3 per cent). The other impurities appeared to be nonhalogenated and aliphatic in nature. These were not further characterized.

All dietary levels of technical pentachlorophenol increased hepatic aryl hydrocarbon hydroxylase activity (15- to 43-fold) (Fig. 1), while 500 ppm of pure pentachlorophenol produced an insignificant 2-fold increase. Technical pentachlorophenol produced significantly greater effects than pure pentachlorophenol at every dietary level. In contrast, neither grade of pentachlorophenol affected aminopyrine *N*-demethylase

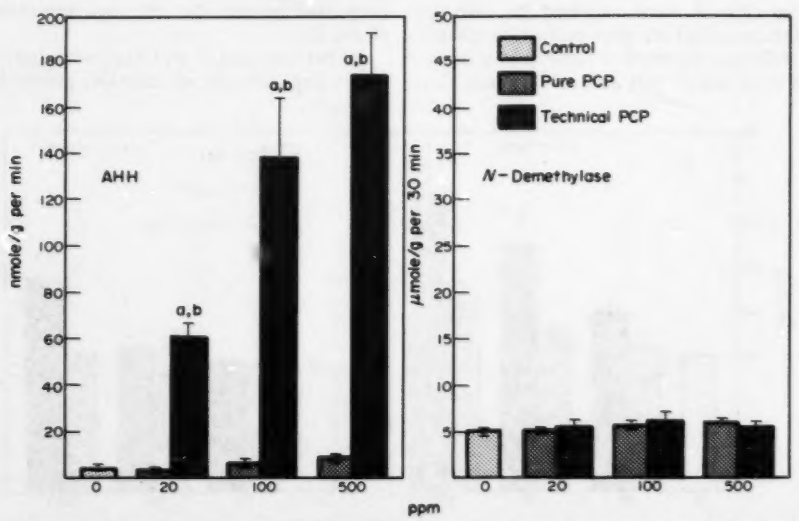

Fig. 1. Effects of technical and pure pentachlorophenol on aryl hydrocarbon hydroxylase and aminopyrine *N*-demethylase. Groups of six female rats were fed 0, 20, 100 or 500 ppm of pure or technical pentachlorophenol and sacrificed at 8 months. Values represent means ± S.E. (a) Significantly greater than controls, $P < 0.05$. (b) Technical pentachlorophenol significantly greater than corresponding dietary level of pure pentachlorophenol, $P < 0.05$.

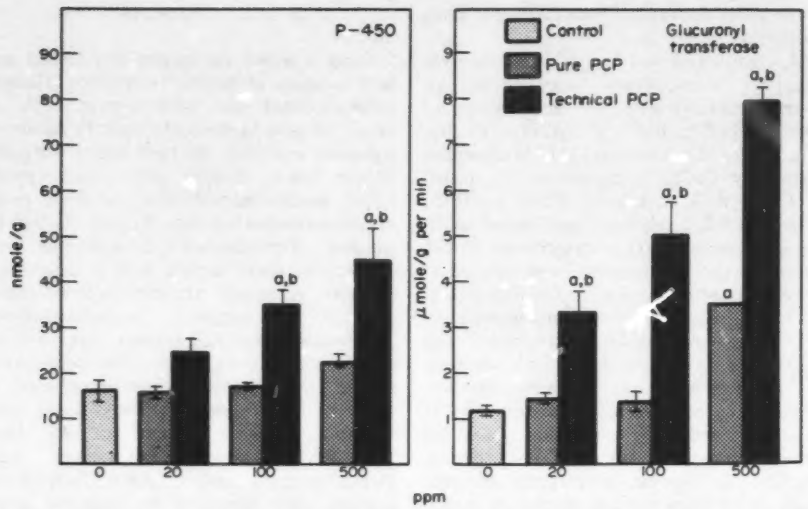

Fig. 2. Effects of technical and pure pentachlorophenol on cytochrome P-450 and glucuronyl transferase. Rats were treated as in Fig. 1. Values represent means ± S.E. (a) Significantly greater than controls, $P < 0.05$. (b) Technical pentachlorophenol significantly greater than the corresponding dietary level of pure pentachlorophenol, $P < 0.05$.

activity. Cytochrome P-450 was increased 2- and 3-fold by 100 and 500 ppm of technical but not pure pentachlorophenol (Fig. 2), and was significantly higher in livers of rats fed technical pentachlorophenol than rats fed pure pentachlorophenol. Microsomal heme paralleled cytochrome P-450 (Fig. 3). Glucuronyl transferase was significantly increased by 20, 100 and 500 ppm of technical pentachlorophenol, but only by 500 ppm of pure pentachlorophenol (Fig. 2). The effect of technical pentachlorophenol was greater than that of pure pentachlorophenol at all dose levels. Liver weight (Table 2) and liver/body weight ratios (Fig. 3) were increased by 100 and 500 ppm of technical but not pure pentachlorophenol.

The CO-difference spectrum of reduced liver microsomes from both control rats and rats fed pure pentachlorophenol peaked at 450 nm. Technical pentachlorophenol shifted the peak of the CO-binding pigment to 448–449 nm. When ethyl isocyanide was used as a ligand, the difference spectra of reduced microsomes from control and pure pentachlorophenol-fed rats were remarkably similar (Fig. 4). The ethyl isocyanide difference spectra from rats treated with technical pentachlorophenol exhibited a shift from 455 to 453 nm, and an increase in absorbance at 453 nm with little or no change in the absorbance at 430 nm. As a result, the ratio of the 455/430 nm peaks was significantly altered by all dietary levels of technical pentachlorophenol, but not pure pentachlorophenol (Table 2).

Urinary porphyrins and ALA were slightly elevated by feeding 500 ppm of technical pentachlorophenol

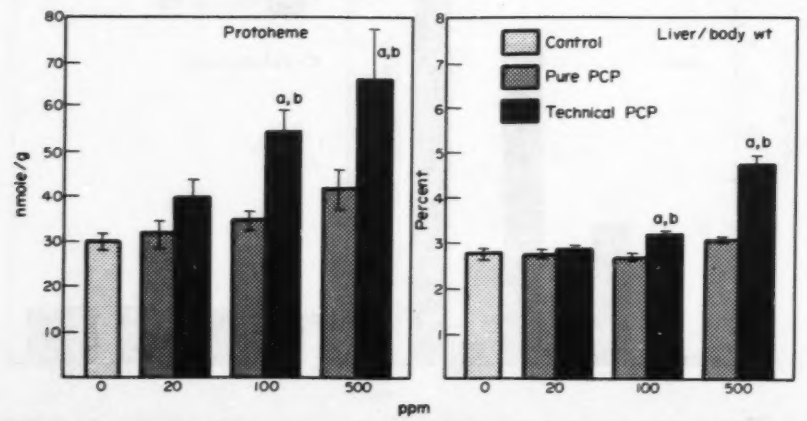

Fig. 3. Effects of technical and pure pentachlorophenol on total microsomal heme and liver/body weight ratios. Rats were treated as in Fig. 1. Values represent means ± S.E. (a) Significantly greater than controls, $P < 0.05$. (b) Technical pentachlorophenol significantly greater than corresponding dietary level of pure pentachlorophenol, $P < 0.05$.

Table 2. Effects of pure and technical pentachlorophenol

Treatment	Ethylisocyanide difference spectra (455/430 nm peak ratio)	Body weight (g)	Liver weight (g)
Control	0.513 ± 0.018	310 ± 14	8.6 ± 0.4
Pure pentachlorophenol (20 ppm)	0.520 ± 0.035	320 ± 14	8.9 ± 0.4
Pure pentachlorophenol (100 ppm)	0.553 ± 0.014	300 ± 15	8.1 ± 0.5
Pure pentachlorophenol (500 ppm)	0.566 ± 0.038	269 ± 10*	8.2 ± 0.2
Technical pentachlorophenol (20 ppm)	0.951 ± 0.143†‡	296 ± 5	8.4 ± 0.4
Technical pentachlorophenol (100 ppm)	1.140 ± 0.10†‡	312 ± 13	9.7 ± 0.6‡
Technical pentachlorophenol (500 ppm)	1.400 ± 0.13†‡	245 ± 7*	11.6 ± 0.3†‡

* Significantly lower than controls, $P < 0.05$.
† Significantly greater than controls, $P < 0.05$.
‡ Technical pentachlorophenol significantly greater than corresponding level of pure pentachlorophenol, $P < 0.05$.

for 1 month, but pure pentachlorophenol had no effect (Table 3). Values remained relatively stable for the next 3 months (data not shown), but after 6 to 8 months of exposure, urinary uroporphyrins rose sharply in two rats fed 500 ppm and one rat fed 100 ppm of technical pentachlorophenol (8-, 10- and 145-fold). At autopsy, total liver porphyrins (Fig. 5) were elevated in one-third of the rats fed 100 and 500 ppm of technical pentachlorophenol (5, 60, 113 and 1287 μg/g vs a 99 per cent confidence limit for controls of < 3.9 μg/g at $P = 0.01$). The livers of several rats fed 500 ppm of pure pentachlorophenol and 100 or 500 ppm of technical pentachlorophenol were totally dark or contained dark areas. Some of these dark areas from livers of rats fed technical pentachlorophenol were fluorescent, and the presence of porphyrins was verified by chemical determination of total porphyrins and thin-layer chromatography. However, some of the dark livers did not fluoresce, and the amount of porphyrin detected by thin-layer chromatography was identical to controls. The porphyrins in porphyric livers were identified as uroporphyrins (20% 7-carboxy- and 80% 8-carboxyporphyrins). Uroporphyrins were not present in detectable amounts in livers from control or pure pentachlorophenol-fed rats. The mean ALA synthetase activity was not affected by pentachlorophenol (Fig. 5), but one value in the group fed 100 ppm of technical pentachlorophenol was outside the normal range (elevated 4-fold), and this liver contained the most porphyrin (1287 μg/g).

Body weight was significantly depressed in rats fed 500 ppm of pure or technical pentachlorophenol (Fig. 6). Food consumption expressed as g/rat/day was approximately equal in all groups, but relative to body weight (g/kg/day) was highest in rats fed 500 ppm of pentachlorophenol. On a mg/kg basis, the dose of pentachlorophenol calculated from the food consumption declined 50 per cent over the 8-month feeding period (Fig. 7).

DISCUSSION

Our results show that chronic exposure to technical pentachlorophenol produces a number of hepatic changes which are consistent with the effects of "biologically active" chlorinated dibenzo-*p*-dioxins. These changes include hepatic porphyria and increases in

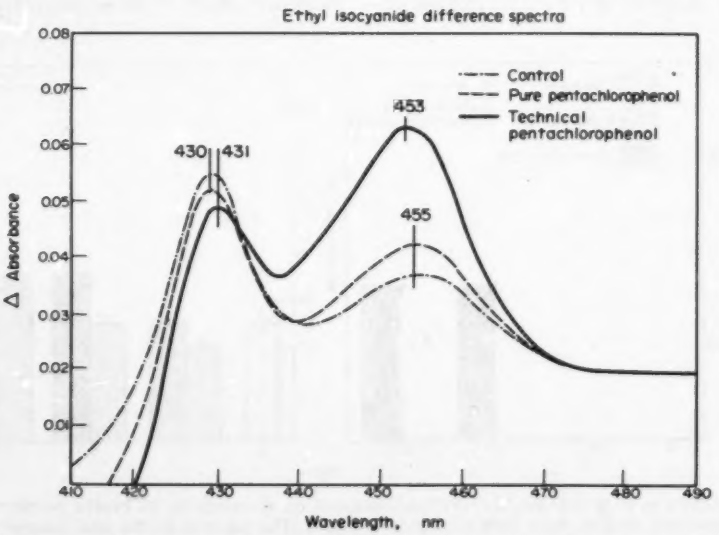

Fig. 4. Ethyl isocyanide difference spectra of microsomes from control rats and rats treated with pure or technical grade pentachlorophenol. Each spectrum represents a single rat fed 500 ppm of pure pentachlorophenol, 500 ppm of technical pentachlorophenol or control chow for 8 months.

Table 3. Effect of pure and technical grades of pentachlorophenol (PCP) on excretion of urinary porphyrins and their precursors*

Treatment	Coproporphyrin		Uroporphyrin		ALA		PBG	
	1 month	8 months	1 month	8 months	1 month	8 months	1 month	8 months
Control	4.3 ± 0.7	4.4 ± 0.5	1.0 ± 0.1	1.0 ± 0.1	16.4 ± 0.7	12.8 ± 0.8	25.5 ± 2.5	27.8 ± 0.6
Pure PCP (20 ppm)	4.1 ± 0.3	3.9 ± 0.5	0.9 ± 0.1	1.5 ± 0.5	15.9 ± 0.9	13.2 ± 1.1	32.5 ± 2.8	29.4 ± 2.0
Pure PCP (100 ppm)	5.9 ± 1.3	5.3 ± 1.2	1.2 ± 0.1	1.0 ± 0.1	15.1 ± 0.8	13.0 ± 0.8	30.1 ± 1.6	28.0 ± 1.3
Pure PCP (500 ppm)	5.4 ± 0.6	4.7 ± 0.8	0.9 ± 0.1	1.0 ± 0.1	17.5 ± 1.1	11.6 ± 0.9	2x.7 ± 1.0	28.0 ± 1.3
Technical PCP (20 ppm)	6.0 ± 1.3	6.6 ± 1.4	0.9 ± 0.1	1.1 ± 0.1	16.6 ± 0.8	14.5 ± 0.7	28.8 ± 0.8	26.6 ± 1.6
Technical PCP (100 ppm)	7.0 ± 1.7	9.6 ± 1.6†‡	1.4 ± 0.3	25.7 ± 24.0§	23.9 ± 3.1†‡	17.7 ± 1.0†‡	41.2 ± 5.1†‡	30.7 ± 1.2
Technical PCP (500 ppm)	12.8 ± 2.7†‡	13.4 ± 2.8†‡	1.7 ± 0.2†‡	4.4 ± 1.5§	33.5 ± 2.1†‡	22.4 ± 1.5†‡	30.7 ± 3.3	38.9 ± 0.7

* Values represent μg excreted/24 hr, expressed as means ± S.E.
† Significantly different from controls, $P < 0.05$.
‡ Significantly greater than corresponding dietary level of pure pentachlorophenol, $P < 0.05$.
§ Means not significantly different from controls because of large S.E., but urinary uroporphyrin excretion of one out of six rats fed 100 ppm and two out of six rats fed 500 ppm of technical pentachlorophenol was greater than the 99 per cent confidence limits for controls (> 3.5 μg/24 hr at $P = 0.01$).

aryl hydrocarbon hydroxylase activity, glucuronyl transferase activity, liver weight, cytochrome P-450 (with a shift of the peak of the CO-difference spectrum from 450 to 448–449 nm), the ratio of the 455:430 nm peaks of the ethyl isocyanide difference spectrum of microsomal hemoprotein, and total microsomal heme, but no increase in aminopyrine *N*-demethylase. Pure pentachlorophenol, in contrast, was not porphyrogenic, had no significant effect on aryl hydrocarbon hydroxylase activity, liver weight, cytochrome P-450, total microsomal heme or the ethyl isocyanide difference spectrum, and only a slight effect on glucuronyl transferase activity.

Similarly, TCDD increases aryl hydrocarbon hydroxylase and glucuronyl transferase activities, but not aminopyrine *N*-demethylase activity [8]. A number of drugs and pesticides induce a variety of drug-metabolizing enzymes, including aminopyrine *N*-demethylase. However, only a few compounds, primarily carcinogenic polycyclic hydrocarbons, induce formation of a cytochrome known as P-448, which differs from cytochrome P-450 by a 2-nm shift in the peak of the CO-difference spectrum, an increase in the ratio of the 455:430 nm peaks of the ethyl isocyanide difference spectrum, and changes in substrate specificity [30]. Recently, TCDD has been shown to have similar effects [7, 24].

The remarkable differences between the hepatic effects of pure and technical pentachlorophenol are consistent with the presence of chlorinated dibenzodioxins and dibenzofurans in technical pentachlorophenol. TCDD was not detected in the technical pentachlorophenol used in this study; however, hexa-, hepta- and octachlorodibenzo-*p*-dioxins, tetra-, penta-, hexa-, hepta- and octachlorodibenzofurans, and predioxins were present. It is unlikely that predioxins or octachlorodibenzo-*p*-dioxin were responsible for the hepatic effects of pentachlorophenol. Octachloro-

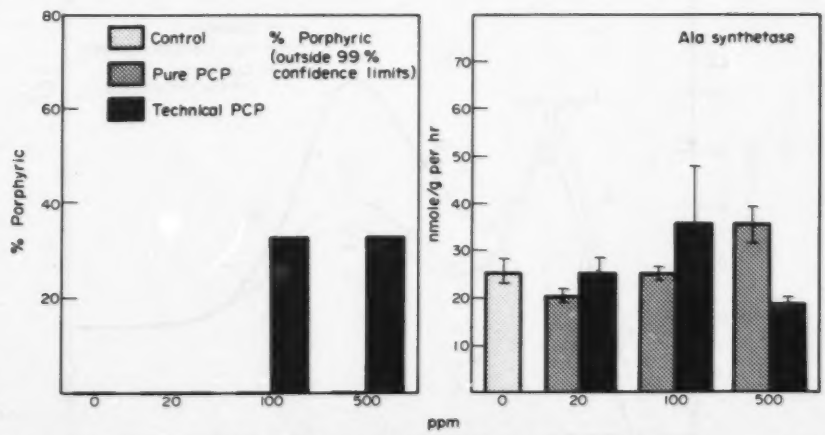

Fig. 5. Effects of pure and technical pentachlorophenol on accumulation of hepatic porphyrins and ALA synthetase activity. Rats were treated as in Fig. 1. The per cent of the total number of rats in each group which are porphyric is shown above (porphyria is defined as a hepatic porphyrin concentration greater than the 99 per cent confidence limit for controls of 3.9 μg/g at $P = 0.01$). ALA synthetase values represent means ± S.E. No treatment groups were significantly greater than controls.

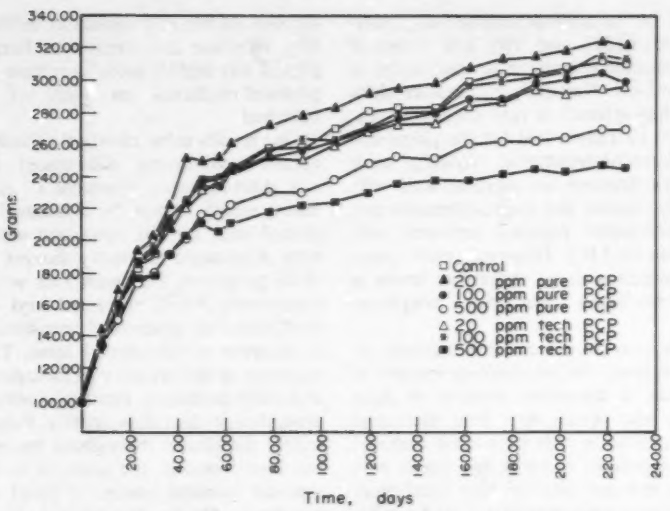

Fig. 6. Effects of pure and technical pentachlorophenol on body weight gain. Rats were treated as in Fig. 1.

dibenzo-*p*-dioxin and predioxins are not lethal at doses 10^4 times the LD_{50} of TCDD [9, 37], nor do they induce aryl hydrocarbon hydroxylase in the chick embryo [38]. On the other hand, although hexa- and heptachlorodibenzo-*p*-dioxins are less potent than TCDD, activity depends largely on the position of the chlorines. Poland and Glover [5] compared the activity of a number of chlorodibenzo-*p*-dioxin isomers as inducers of aryl hydrocarbon hydroxylase and ALA synthetase in the chick embryo,

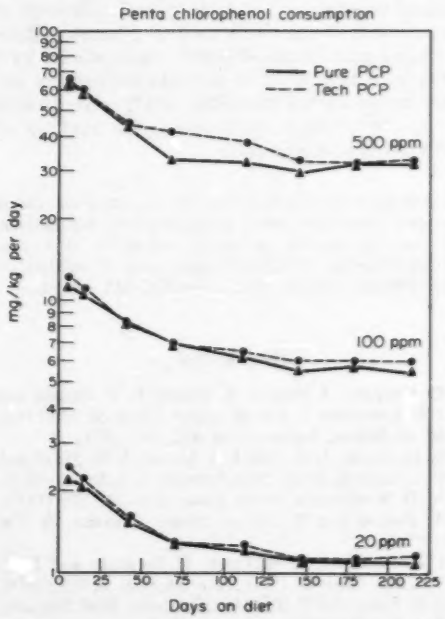

Fig. 7. Consumption of pentachlorophenol by rats fed diets containing pure and technical pentachlorophenol. The rats were treated as in Fig. 1.

and Bradlaw *et al.* [39] compared activity as inducers of aryl hydrocarbon hydroxylase in rat hepatoma cells in culture. Hexa- and hepta-isomers with the four lateral ring positions (2,3,7,8-) occupied by chlorines had considerable activity. The structure–activity relationship of the chlorinated dibenzofurans appears to be similar to that of the chlorinated dibenzo-*p*-dioxins [38]. The relative potency of most of the chlorinated dibenzo-*p*-dioxin and dibenzofuran isomers *in vivo* is not known; however, the relative potency of those isomers which have been tested *in vivo* correlated well with the potency *in vitro* [9, 37].

From the food consumption, we calculated that rats fed 20 ppm of technical pentachlorophenol, a dose which increased aryl hydrocarbon hydroxylase activity, received 10–20 ng/kg/day of hexa-, 0.6 to 1.3 μg/kg/day of hepta-, and 1.6 to 3.3 μg/kg/day of octachlorodibenzo-*p*-dioxins, as well as 5–10 ng/kg/day of tetra-, 50–100 ng/kg/day of penta-, 225–450 ng/kg/day of hexa-, 1–2 μg/kg/day of hepta- and 0.65 to 1.3 μg/kg/day of octachlorodibenzofurans. The dose of dioxins and furans consumed by rats fed 100 ppm of technical pentachlorophenol, a dose which produced porphyria, was five times higher than the above values. In comparison, single doses of 200 ng/kg of TCDD increase aryl hydrocarbon hydroxylase activity. Because of the long half-life (17 days) and high liver distribution of TCDD [40], we would expect the effects of chronic dosing to be cumulative. Aryl hydrocarbon hydroxylase is increased by sixteen weekly doses of 10 ng/kg/week of TCDD, and porphyria occurs after sixteen weekly doses of 0.1 μg/kg/week [41]. These data are compatible with the hypothesis that the hepatic effects of technical pentachlorophenol reported in this paper are due to the presence of chlorinated dibenzo-*p*-dioxins and dibenzofurans at the concentrations reported.

The type of porphyria induced by technical pentachlorophenol, characterized by delayed onset after exposure, and increased excretion and hepatic accumulation of uroporphyrins, is similar to the por-

phyria produced by hexachlorobenzene [42], polychlorinated biphenyls [32] and very low doses of TCDD [41]. Pentachlorophenol is a metabolite of hexachlorobenzene [43]. However, our results show that pure pentachlorophenol is not porphyrogenic; therefore, it cannot be responsible for the porphyrogenic effects of hexachlorobenzene. Notably, some grades of hexachlorobenzene are contaminated with octachlorodibenzo-*p*-dioxin and chlorodibenzofurans, and some polychlorinated biphenyl mixtures with tetrachlorodibenzofuran [44]. However, pure hexachlorobiphenyl isomers with no detectable levels of chlorinated dibenzofurans are also porphyrogenic [45].

Technical pentachlorophenol did not increase activity of ALA synthetase, the rate-limiting enzyme in porphyrin synthesis. A detectable increase in ALA synthetase activity also occurs later than the initial rise in hepatic porphyrins in polychlorinated biphenyl poisoning [32]. The primary defect in this type of porphyria is probably not induction of ALA synthetase.

Pure and technical pentachlorophenol had similar effects on only two parameters. First, both pure and technical pentachlorophenol decreased body weight gain comparably in the absence of an effect on food consumption, suggesting poor utilization of food at the 500 ppm level. It is probable that the decrease in body weight is related to the fact that pentachlorophenol is a known uncoupler of oxidative phosphorylation [46]. Second, the livers of some rats fed both pure and technical pentachlorophenol were discolored in the absence of any accumulation of porphyrins. The identity of the pigment is not known.

Few studies have explored the possibility that the biological effects of pentachlorophenol could be produced by the highly active contaminants present in varying amounts in various commercial preparations. Johnson *et al.* [47] reported that a commercial grade of pentachlorophenol contaminated with 20 ppm hexa- and 2000 ppm octachlorodibenzo-*p*-dioxin was more toxic than pure pentachlorophenol. Technical pentachlorophenol produced a positive response in the chick edema and rabbit ear bioassays, elevated serum alkaline phosphatase, decreased serum albumin, hemoglobin and packed cell volume, and produced minimal hepatocellular degeneration and necrosis in rats, while pure pentachlorophenol with no detectable concentrations of chlorinated dibenzo-*p*-dioxins produced none of these effects. Kimbrough and Linder [20] found more histological changes in livers of rats treated with technical pentachlorophenol than rats treated with pure pentachlorophenol. On the other hand, Schwetz *et al.* [48] compared purified and commercial grades of pentachlorophenol on embryonic and fetal development in the rat and concluded that the amounts of nonphenolics in technical pentachlorophenol (including 4 ppm hexa-, 125 ppm hepta-, and 2500 ppm octachlorodibenzo-*p*-dioxins, 30 ppm hexa-, 80 ppm hepta- and 80 ppm octachlorodibenzofurans) did not contribute significantly to the effects of this material in developing rat embryo and fetus. They found that 15, 30 or 50 mg/kg/day of pure pentachlorophenol given during early organogenesis caused a significant increase in the incidence of resorptions, a decrease in maternal and fetal body weight, a decrease in fetal crown–rump length, subcutaneous edema and variations in the development of ribs, vertebrae and sternebrae. Purified pentachlorophenol was slightly more toxic than commercial grade pentachlorophenol on each of the parameters observed.

Our results show clearly that technical pentachlorophenol containing chlorinated dibenzo-*p*-dioxins and dibenzofurans produces a number of hepatic effects which cannot be attributed to pentachlorophenol itself, but are consistent with the effects seen with chlorinated dibenzo-*p*-dioxins. These effects include porphyria, increased liver weight, induction of cytochrome P-448, increased aryl hydrocarbon hydroxylase and glucuronyl transferase activities, and an increase in microsomal heme. The most sensitive indicator of the presence of biologically active dioxin and furan isomers in pentachlorophenol was aryl hydrocarbon hydroxylase activity. Pentachlorophenol is widely distributed throughout the environment, and has been found in the urine of both occupationally exposed workers (mean, 20 ppm) and the general population (mean, 40 ppb) [49]. It should be noted that some commercial grades of pentachlorophenol have much lower concentrations of chlorinated dibenzo-*p*-dioxins and dibenzofurans than the grade utilized in the present study [47]. However, the magnitude of the effects seen with 20 ppm of technical pentachlorophenol (contaminated with 8 ppm hexa-, 520 ppm hepta- and 1400 ppm of octachlorodibenzo-*p*-dioxin as well as 4 ppm tetra-, 40 ppm penta-, 90 ppm hexa, 400 ppm hepta and 260 ppm of octachlorodibenzofurans) and the difficulty in quantitating chlorinated dibenzo-*p*-dioxins and dibenzofurans in the environment at ppt levels emphasize the importance of controlling the quality of pesticides and herbicides. Moreover, this study points out the importance of determining the quantity and toxicology of contaminants of chemicals used in pharmacological or toxicological studies. Possibly, many studies have attributed biological effects to pentachlorophenol and other environmental chemicals which were actually due to the presence of undetermined amounts of highly active contaminants.

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REFERENCES

1. D. Firestone, J. Ress, N. L. Brown, R. P. Barron and J. N. Darminco, *J. Ass. off. analyt. Chem.* **55**, 85 (1972).
2. M. H. Milnes, *Nature, Lond.* **232**, 395 (1971).
3. B. N. Gupta, J. G. Vos, J. A. Moore, J. G. Zinkl and B. C. Bullock, *Envir. Hlth Perspect. Exp.* **5**, 125 (1973).
4. R. D. Kimbrough, *Archs Envir. Hlth* **25**, 125 (1972).
5. A. Poland and E. Glover, *Molec. Pharmac.* **9**, 736 (1973).
6. J. A. Goldstein, P. Hickman, H. Bergman and J. G. Vos, *Res. Commun. Chem. Path. Pharmac.* **6**, 919 (1973).
7. J. B. Greig and F. De Matteis, *Envir. Hlth Perspect. Exp.* **5**, 211 (1973).
8. G. W. Lucier, O. S. McDaniel, G. E. R. Hook, B. A. Fowler, B. R. Sonawane and E. Faeder, *Environ. Hlth Perspect. Exp.* **5**, 199 (1973).

9. B. A. Schwetz, J. M. Norris, G. L. Sparchu, V. K. Rowe, P. J. Gehring, J. L. Emerson and C. G. Gerbig, *Adv. Chem. Ser.* **121**, 55 (1973).
10. J. Bleiberg, M. Wallen, R. Brodkin and J. L. Applebaum, *Archs Derm.* **89**, 793 (1964).
11. A. Poland, D. Smith, G. Metter and P. Possick, *Archs Envir. Hlth* **22**, 316 (1971).
12. H. C. E. W. Baader and H. J. Bauer, *Ind. Med. Surg.* **20**, 286 (1951).
13. J. Kimming and K. H. Schulz, *Dermatologica* **115**, 540 (1957).
14. J. S. Cantrell, N. C. Webb and A. J. Mabis, *Acta crystallogr.* **B25**, 150 (1969).
15. L. D. Metcalfe, *J. Ass. off. analyt. Chem.* **55**, 542 (1972).
16. C. Rimington and G. Ziegler, *Biochem. Pharmac.* **12**, 1387 (1963).
17. C. Cam, *Dirim.* (Istanbul, in Turkish) **34**, 11 (1959).
18. E. C. Villanueva, R. W. Jennings, V. W. Burse and R. D. Kimbrough, *J. agric. Fd Chem.* **22**, 916 (1974).
19. I. Knudsen, H. G. Verschuuren, E. M. Den Tonhelaar, R. Kroes and P. F. W. Helleman, *Toxicology* **2**, 141 (1974).
20. R. D. Kimbrough and R. E. Linder, *Toxic. appl. Pharmac.* **33**, 131 (Abstr.) (1975).
21. W. B. Crummett and R. H. Stehl, *Envir. Hlth Perspect. Exp.* **5**, 15 (1973).
22. H. S. Marver, D. P. Tschudy, M. G. Perlroth and A. Collins, *J. biol. Chem.* **241**, 2803 (1966).
23. J. R. Fouts, *Toxic. appl. Pharmac.* **16**, 48 (1970).
24. A. Poland and G. Glover, *Molec. Pharmac.* **10**, 349 (1974).
25. D. L. Cintini, P. Moldeus and J. B. Schenkman, *Biochem. Pharmac.* **21**, 3249 (1972).
26. J. A. Goldstein and A. Taurog, *Biochem. Pharmac.* **17**, 1049 (1968).
27. G. W. Lucier, O. S. McDaniel and H. G. Matthews, *Archs Biochem. Biophys.* **145**, 520 (1971).
28. T. Omura and R. Sato, *J. biol. Chem.* **239**, 2370 (1964).
29. T. Omura and R. Sato, *J. biol. Chem.* **239**, 2379 (1964).
30. N. E. Sladek and G. J. Mannering, *Biochem. biophys. Res. Commun.* **24**, 668 (1966).
31. O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. biol. Chem.* **193**, 265 (1951).
32. J. A. Goldstein, P. Hickman and D. L. Jue, *Toxic. appl. Pharmac.* **27**, 437 (1974).
33. H. S. Marver, D. P. Tschudy, M. G. Perlroth, A. Collins and G. Hunter, Jr., *Analyt. Biochem.* **14**, 53 (1966).
34. G. Abbritti and F. De Matteis, *Chem. Biol. Interact.* **4**, 281 (1971–1972).
35. M. Doss, *J. Chromat.* **30**, 265 (1967).
36. R. G. D. Steel and J. H. Torrie, in *Principles and Procedures of Statistics*, pp. 107–9. McGraw-Hill, New York (1960).
37. E. E. McConnell and J. A. Moore, *Toxic. appl. Pharmac.* **35** (abstr.) 146 (1976).
38. A. Poland, E. Glover and A. S. Kende, *J. biol. Chem.* **251**, 4936 (1976).
39. J. A. Bradlaw, L. H. Barthoff, N. E. Hurley and D. Firestone, *Toxic. appl. Pharmac.* **35**, 119 (abstr.) (1976).
40. W. N. Piper, J. Q. Rose and P. J. Gehring, *Adv. Chem. Ser.* **120**, 85 (1973).
41. J. A. Goldstein, P. Hickman and H. Bergman, *Fedn Proc.* **35** (abstr.) 708 (1976).
42. R. K. Ockner and R. Schmid, *Nature, Lond.* **189**, 499 (1961).
43. H. Lui and G. D. Sweeney, *Fedn Eur. Biochem. Soc. Lett.* **51**, 225 (1975).
44. G. W. Bowes, M. J. Mulvihill, B. R. T. Simoneit, A. L. Burlingame and R. W. Risebrough, *Nature, Lond.* **256**, 305 (1975).
45. J. A. Goldstein, J. D. McKinney, G. W. Lucier, J. A. Moore, P. Hickman and H. Bergman, *Pharmacologist* **16** (abstr.) 239 (1974).
46. E. C. Weinbach, *Proc. natn. Acad. Sci. U.S.A.* **43**, 393 (1957).
47. R. L. Johnson, P. J. Gehring, R. J. Kociba and B. A. Schwetz, *Envir. Hlth Perspect. Exp.* **5**, 171 (1973).
48. B. A. Schwetz, P. A. Keeler and P. A. Gehring, *Toxic. appl. Pharmac.* **28**, 151 (1974).
49. A. Bevenue, J. Wilson, L. J. Casarett and H. W. Klemmer, *Bull. envir. Contam. Toxic.* **2**, 319 (1967).